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Ozone effects on visible foliar injury and growth of *Fagus sylvatica* and *Viburnum lantana* seedlings grown in monoculture or in mixture

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Abstract

Seedlings of *Fagus sylvatica* (beech) and *Viburnum lantana* (Viburnum) grown in monoculture and mixture were exposed to ambient and sub-ambient (charcoal-filtered) ozone concentrations in open-top chambers over the course of the 2003 and 2004 growing seasons at the WSL Lattecaldo open-top chamber facility in southern Switzerland. The aim of the study was to determine how the sensitivity to ozone in ambient air of these two species would differ between monocultures and mixtures in terms of growth and visible foliar injury development. Ambient ozone concentrations were consistently higher from the end of April to the middle of October in 2003 than in 2004 with seasonal peaks and means reaching 147 and 50 parts-per-billion (ppb) in 2003 compared to 124 and 40 ppb in 2004. Ambient AOT40 (ozone concentration accumulated over a threshold of 40 ppb during daylight hours with global radiation >50 W m^{-2}) values from the end of April to the middle of October reached 48.3 and 26.8 parts-per-billon hours (ppm h) in 2003 and 2004, respectively. In general, Viburnum was a stronger competitor than beech over the course of this 2-year study. Seedlings of Viburnum benefited from interspecific competition in terms of both height growth and above-ground biomass accumulation at the expense of beech seedlings, which showed significantly reduced growth in the mixture as compared to the monoculture. However, as this was only the case for Viburnum growing in the charcoal-filtered treatment, ozone seemed to counteract the beneficial effect of interspecific competition on above-ground biomass accumulation in Viburnum, while at the same time decreasing relative biomass allocation to roots. Foliar sensitivity of the two species was also altered under interspecific competition suggesting that results based on seedlings of single species grown in monocultures may significantly over- or under-estimate foliar sensitivity to ozone. These results demonstrate that competition is an important factor affecting plant responses to ozone stress, but the direction and severity of these effects depend on the interacting species.

Keywords: Ozone; Competition; Visible injury; Growth; Open-top chambers; Southern Switzerland

1. Introduction

Over the past several decades, ozone air pollution effects on forests have been the focus of numerous studies across North America and Europe. As a result, the effects of ozone at the biochemical, cellular, physiological, and morphological level have been well characterized for a variety of forest species grown in a wide range of experimental and natural conditions (Pye, 1988; Matyssek and Sandermann, 2003). However, despite decades of research on this topic, there are still gaps of knowledge in our understanding of ozone impacts at the forest ecosystem level (Matyssek and Innes, 1999). This is partly because the majority of information available on ozone effects on trees was derived from experiments with seedlings in controlled or semi-controlled conditions (i.e., growth chambers or open-top chambers) often maintained at non-limiting conditions for growth and ozone uptake, making the extrapolation of these results to actual forest conditions rather difficult (Kolb et al., 1997). In addition, many of the studies were conducted using single species grown

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in a non-competitive environment, whereas, the ozone sensitivity of the very same species may be altered significantly when grown in mixture with other species (Kozovits et al., 2005a).

Several recent studies have investigated the potential effects of competition on the ozone sensitivity of forest trees. Andersen et al. (2001) found that ponderosa pine seedlings were more sensitive to ozone when grown in competition with blue wild-rye grass. Similarly, a 2-year study exposing seedlings of loblolly pine grown in an early successional plant community to four ozone treatments found that height, diameter and volume growth were significantly negatively correlated to percent total vegetative cover (Barbo et al., 2002). McDonald et al. (2002), investigating competition effects on growth of individual trees in aggrading, mixed-clone stands of trembling aspen exposed to elevated ozone and CO_2, found that the magnitude of the ozone response was dependent on the clone as well as on the competitive status. Recently in Europe, juvenile European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.) grown under intra- and inter-specific competition were exposed to factorial combinations of ambient and elevated ozone and CO_2 in a 2-year phytotron study (Grams et al., 2002; Liu et al., 2004; Kozovits et al., 2005a,b). Spruce was generally a stronger competitor than beech and appeared to benefit from interspecific competition with beech, which was mainly due to its superior ability in above-ground space sequestration (Grams et al., 2002; Kozovits et al., 2005b). However, spruce was generally unaffected by increased ozone concentrations in either competitive environment. Beech was at a competitive disadvantage when growing in mixture with spruce as reflected by reduced leaf gas exchange, biomass development and crown volume, and this disadvantage was enhanced under increased ozone concentrations (Kozovits et al., 2005a). On the contrary, Liu et al. (2004) found that the ozone sensitivity of beech was reduced in terms of carbohydrate accumulation when grown in competition with spruce as compared to the beech in monoculture. These results illustrate the genotypic and species-specific variation in responses to elevated ozone exposures and competition as well as the dependency of these responses on the type of competition involved, i.e., intraspecific versus interspecific.

In the current study, seedlings of *F. sylvatica* (beech) and *Viburnum lantana* L. (Viburnum) grown in monoculture and mixture were exposed to ambient and sub-ambient ozone concentrations in open-top chambers over the course of two growing seasons. The choice of these two species was based on both their contrasting growth strategies as well as on their known contrasting sensitivity to ozone. Viburnum is a common European shrub species that grows under a range of light and edaphic conditions (Kollmann and Grubb, 2002) and shows a generally continuous growth pattern throughout the season, often producing multiple stems. Beech is also a widespread tree species across Europe, but in contrast to Viburnum, is generally slower growing with a determinant growth pattern. Previous studies have also determined Viburnum to be more sensitive to ozone exposures than beech in terms of the severity of ozone-induced visible foliar injury as well as the timing of injury onset (VanderHeyden et al., 2001). Based on the inherently different growth and physiology of the two species as well as the findings of previous competition studies involving beech (see above), we hypothesized that Viburnum would be a stronger competitor than beech when grown under interspecific competition. Furthermore, we aimed to determine how the sensitivity to ozone in ambient air of these two species would differ between monocultures and mixtures in terms of growth and visible foliar injury development.

2. Materials and methods

2.1. Site characteristics and experimental design

The 2-year study was conducted at an open-top chamber facility located in the pre-alpine region of southern Switzerland within the Lattecaldo Cantonal Forest Nursery in Valle di Muggio, Canton Ticino (09°03′E, 45°51′N, 600 m above sea level) (Skelly et al., 1998, 1999; VanderHeyden et al., 2001; Novak et al., 2003, 2005). The experimental design consisted of two ozone treatments in open-top chambers (OTCs) with four replications (8 plots; $n = 4$): four non-filtered (NF) OTCs with approximately 93% of the ambient ozone concentrations and four charcoal-filtered (CF) OTCs with approximately 47% of the ambient ozone concentrations. Within each OTC, fiberglass separations were installed 50 cm into the soil to divide the plot into equal thirds. Separations were also installed in the soil around the perimeter of each plot to exclude the surrounding grass roots. The nursery soil type was a well-tilled silicate limestone-derived calcaric regosol (Zhang et al., 2001). In mid-April of 2003, each third was planted with 28 seedlings arranged in a grid design using 20 cm spacing between plants. The seedlings were planted in four rows with the first and fourth rows containing six individuals each and the second and third rows containing eight individuals each. One section of the chamber was planted with a monoculture of 4-year-old *F. sylvatica* L. (European beech) seedlings, a second section with a monoculture of 2-year-old *V. lantana* L. seedlings and the third section with a 1:1 mixture of the two species (14 seedlings of beech and 14 seedlings of Viburnum). As Viburnum is a faster growing species than beech, 2-year-old seedlings were used to avoid height dominance by Viburnum in the establishment stage of the experiment. All seedlings were derived from seed collected from parent plants near the Lattecaldo nursery.

Following planting, the OTC plastic panels and charcoal filters were installed and the ozone treatments began in the end of April 2003. The plastic panels and charcoal filters were removed during winter and installed again prior to the start of the growing season in mid-April 2004. To minimize edge effects, all measurements and assessments focused on the inner eight individuals of each section, only. All plots were watered routinely throughout the experiment to minimize water stress to the plants; no fertilizer was added.

2.2. Ozone and meteorological measurements

Ozone concentrations in parts-per-billion (ppb) were continuously monitored throughout the 2003 and 2004 growing seasons from 21 April to 18 October using a Monitor Labs model ML 8810 ozone monitor, which was calibrated monthly. Two-minute air samples were drawn at 1 m height from each OTC and one ambient plot, following a repeating 20 min sampling interval. Meteorological data were also collected throughout each growing season beginning in May 2003 from an on-site measurement station located next to the OTCs. Hourly measurements included air temperature (°C), relative humidity (%), and global radiation ($W m^{-2}$).

2.3. Visible foliar injury assessment

Visible foliar injury was assessed regularly from leaf expansion in the beginning of May to mid-October in 2003 and 2004. Symptoms on plants growing in NF treatment were compared to plants growing in the CF chambers to confirm ozone as the cause of foliar symptoms. All plants were observed macroscopically; potential ozone-induced symptoms were further confirmed using a 10× hand lens. A 5% scale (0, 5, 10, 15...100%) was used to estimate the percentage of symptomatic leaves per plant and the Horsfall–Barratt scale (0, 1, 3, 6, 12, 25, 50, 75, 88, 94, 97, 99, and 100%) was used to estimate the average percentage of area injured on symptomatic leaves (Horsfall and Barratt, 1945). The estimated values were then multiplied by one another and divided by 100 to obtain the estimated percentage of total leaf area injured by ozone for each plant. The dates of the onset of ozone-induced visible injury were recorded for each plant during both growing seasons. The same observer made all assessments during both growing seasons.

2.4. Growth and biomass assessments

The heights of the seedlings were measured four times throughout the 2003 growing season on 6 May, 3 July, 15 September, and 17 October and five times in 2004 on 20 April, 19 May, 19 June, 29 August, and 26 October. Heights were measured from the soil surface to the base of the highest bud of the tallest stem. Beech seedlings only produced one main stem while Viburnum seedlings often produced multiple stems from the base, and therefore, the number of stems for Viburnum was recorded twice in 2004 on 19 June and 26 October.

All seedlings were harvested in November 2004 at the conclusion of the experiment for the determination of non-green above-ground biomass (i.e., excluding leaves). Seedlings were cut at the soil surface, separated into stems and branches, and dried at 70 °C until constant weight. Soil cores were also taken at the same time for determination of relative root biomass (grams of root dry mass per kilograms of soil dry mass) for each section and OTC. Three representative cores from in between the inner eight seedlings were taken from each beech monoculture, Viburnum monoculture, and mixed culture; roots could not be separated by species in the mixed culture. The roots were separated from the soil and both were dried at 70 °C until constant weight.

2.5. Data analysis

Data were checked for normality and homogeneous variance prior to statistical analysis and log-transformed when necessary. A split–plot analysis of variance (ANOVA) was conducted for above-ground biomass parameters at the end of the second season and seedling heights at the end of the both seasons with ozone treatment as the main plot and type of competition (intra- and inter-specific) as the subplot to test for significant ozone treatment effects, effects of growth in mixture, and their interaction. Differences in means of root biomass between treatments were determined using a two-sided, unpaired Wilcoxon rank-sum test. Due to a significant deviation from normality in the foliar injury data, the non-parametric Kruskal–Wallis test was used to test for significant differences in foliar injury between ozone treatments and between monoculture and mixture. A significance level of 0.05 was used for all tests. All statistical analyses were performed using the Statistical Analysis System (SAS) (SAS Institute Inc., 1999).

3. Results

3.1. Ozone exposures and climatic conditions

Monthly mean and 1 h peak ozone concentrations as well as monthly mean temperature and relative humidity are summarized in Table 1. The 2003 growing season in southern Switzerland, as well as much of Europe, was extreme in terms of both ozone exposures and temperatures, while 2004 was within the normal range of conditions that would be expected at the study site. Seasonal daily mean temperature was 3 °C greater and mean RH was 6% less in 2003 than in 2004. Ambient ozone concentrations were consistently higher from the end of April to the middle of October in 2003 than in 2004 with seasonal peaks and means reaching 147 and 50 ppb in 2003 compared to 124 and 40 ppb in 2004. AOT40 (ozone concentration accumulated over a threshold of 40 ppb during daylight hours with global radiation >50 $W m^{-2}$) values from the end of April to the middle of October reached 48.3, 41.5 and 8.2 ppm h in the AA, NF and CF, respectively, in 2003 and 26.8, 21.8 and 4.1 ppm h, respectively, in 2004 (Fig. 1).

3.2. Visible foliar injury

Ozone-induced visible foliar injury was observed on both beech and Viburnum in the NF treatment in both 2003 and 2004 (Table 2 and Fig. 2). Foliar injury on the leaves of Viburnum was characterized as fine reddish upper surface, interveinal stipple. As the injury progressed, the reddish stipple covered much of the upper leaf surfaces. Foliar injury on the leaves of beech was characterized as very fine but uniform upper surface brown stipple; a bronzed appearance sometimes occurred simultaneously. The percentage of injured plants, date of first symptom and mean injury onset dates for mixed and monocultures in 2003 and 2004 are summarized in Table 2. In general, a greater percentage of Viburnum plants were injured and injury onset was earlier as compared to beech in both 2003 and 2004. For beech in 2003, a greater percentage of plants showed ozone-induced injury in the monoculture versus the mixed culture, but the mean injury onset date was earlier in the mixed culture than in the monoculture. In 2004, a greater percentage of injured beech plants were observed in the monoculture versus mixed

Table 1
Monthly and seasonal 24 h mean (μ) and hourly peak ozone concentrations in the ambient (AA), non-filtered (NF), and charcoal-filtered (CF) air treatments and the 24 h mean ambient air temperature (Temp) and relative humidity (RH) measured from the end of April to the middle of October in 2003 and 2004 at the Lattecaldo OTC facility, Switzerland

	Ozone (ppb)						Temp (°C)	RH (%)
	AA		NF		CF			
	μ	Peak	μ	Peak	μ	Peak		
April[a]								
2003	45	80	41	72	19	45	n.a.	n.a.
2004	40	79	36	71	19	49	13	60
May								
2003	47	102	44	96	21	63	17	55
2004	41	93	38	86	20	61	13	67
June								
2003	63	145	59	140	31	101	23	59
2004	48	112	44	103	24	73	19	59
July								
2003	62	138	59	130	29	85	22	60
2004	56	124	52	122	27	80	20	60
August								
2003	62	147	58	139	27	86	24	57
2004	46	108	43	99	22	67	20	64
September								
2003	46	126	42	120	20	72	16	60
2004	34	95	31	88	15	52	17	65
October[b]								
2003	23	52	21	47	10	37	12	65
2004	18	49	17	47	8	30	12	80
Season mean								
2003	50	147	46	140	22	101	19	59
2004	40	124	37	122	19	80	16	65

[a] O_3 data collection began on 21 April in both years.
[b] O_3 data collection ended on 18 October in both years.

Table 2
Percentage of plants showing ozone-induced visible foliar injury, date of first ozone-induced symptom, and average injury onset for seedlings of beech (*Fagus sylvatica*) and Viburnum (*Viburnum lantana*) grown in the non-filtered air (NF) treatment in monocultures and mixed cultures (mean, μ, and standard error, S.E.)

	Year	% of plants injured	Date of first symptom	Average injury onset	
				μ	S.E.
Beech					
Mono	2003	66	28 June	6 August	7
	2004	44	21 July	13 August	4
Mixed	2003	47	28 June	13 July	6
	2004	13	21 July	14 August	24
Viburnum					
Mono	2003	68	28 June	14 August	8
	2004	75	12 May	19 May	3
Mixed	2003	69	19 June	14 July	7
	2004	94	12 May	21 May	4

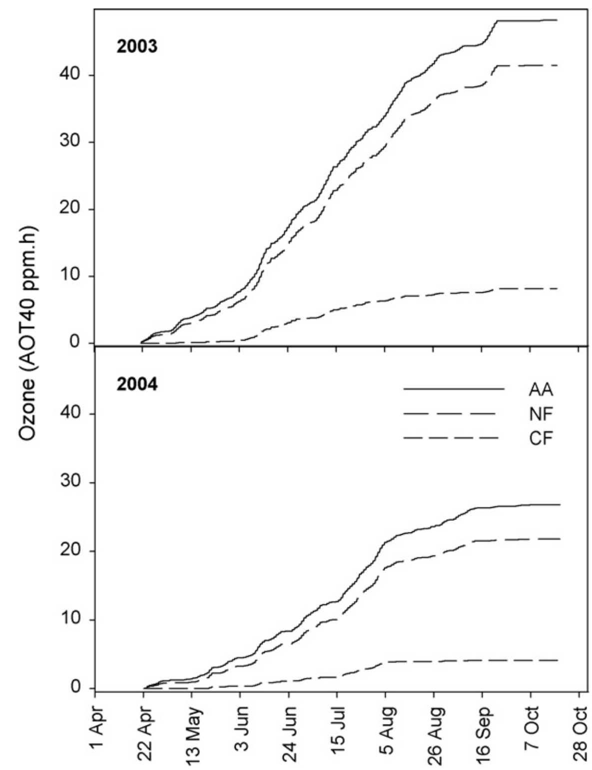

Fig. 1. Ozone exposures (AOT40 ppm h) (accumulated over threshold of 40 ppb during daylight hours >50 W m^{-2}) for ambient (AA), non-filtered (NF), and charcoal-filtered (CF) air treatments at the Lattecaldo OTC facility in 2003 and 2004.

culture, however, there was no difference in injury onset dates between the two types of cultures. No difference was observed in the percentage of injured Viburnum plants in 2003, but as with beech, injury onset was earlier in the mixed culture than in the monoculture. In 2004, a greater percentage of Viburnum plants were injured in the mixed culture versus monoculture with no significant differences in injury onset dates.

Beech showed more severe ozone-induced visible foliar injury than Viburnum in 2003 and although not statistically significant, there was a tendency towards greater injury severity in the mixed culture versus monoculture in both species at the end of the observation period (Fig. 2 and Table 3). On the contrary, Viburnum showed greater injury severity than beech in 2004 (Fig. 2). In addition, there were significant differences between cultures with Viburnum exhibiting more injury in the mixed culture than in the monoculture, whereas beech showed the opposite with greater injury in the monoculture versus mixed culture (Fig. 2 and Table 3).

3.3. Height and stem growth

There were no significant effects of ozone or mixture observed on the height of beech seedlings in either 2003 or 2004 (Table 3 and Fig. 3). Viburnum also showed no significant ozone effects on height growth at the end of the season in either year. However, a significant effect of mixture on height growth of Viburnum was observed at the end of the season in 2003, which persisted throughout the 2004 growing season (Table 3 and

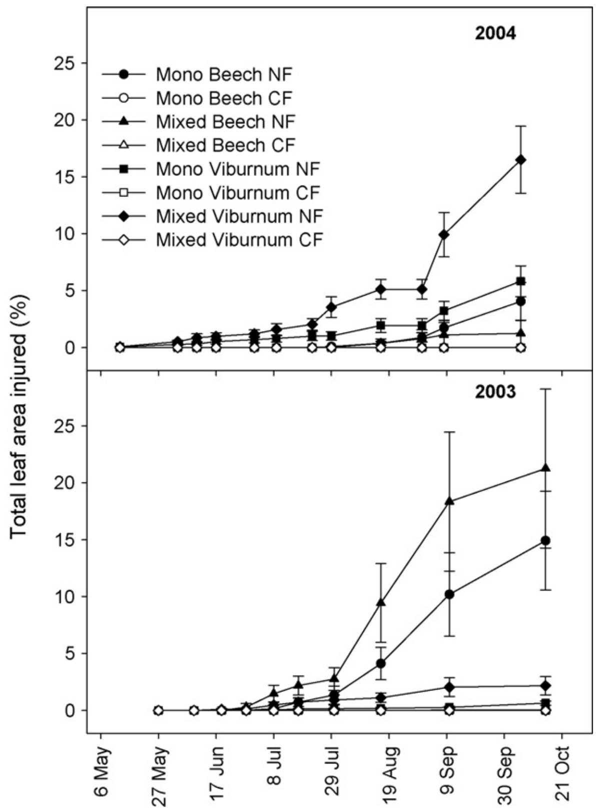

Fig. 2. Mean and standard error of total leaf area injured per plant for beech (*Fagus sylvatica*) and Viburnum (*Viburnum lantana*) grown in non-filtered (NF) and charcoal-filtered (CF) air treatments in monocultures and mixed cultures during 2003 and 2004.

Fig. 3). On average, Viburnum seedlings were approximately 30 cm taller than beech in the mixed culture at the conclusion of the experiment (Fig. 3). There was no significant ozone effect on number of stems in Viburnum, but there were slightly more stems observed in the monoculture versus mixed culture at the conclusion of the experiment.

3.4. Above-ground biomass

In general, Viburnum had a greater total above-ground biomass than beech at the conclusion of the experiment (Fig. 4A). Significant differences between CF and NF in total above-ground biomass were not observed in either species (Table 3 and Fig. 4A). However, significant differences between monoculture and mixture in total above-ground biomass were observed in both species with Viburnum showing an increase in total above-ground biomass in the mixed culture at the expense of beech, which had a reduced biomass in the mixed culture compared to the monoculture. A significant effect of growth in mixture was also observed on branch biomass, which followed a similar trend with increased biomass in the mixed culture for Viburnum and decreased biomass in the mixed culture for beech. In addition, there was a significant interaction between ozone treatment and growth in mixture as shown by a significant negative ozone effect on branch biomass for Viburnum growing in the mixture with beech, whereas no significant difference between CF and NF was observed in the Viburnum monoculture (Fig. 4B and Table 3). When comparing ratios of branch biomass to total above-ground biomass, there were no significant ozone treatment effects in either culture or mixture effects observed for beech. In Viburnum, on the other hand, there was a significant ozone treatment effect in the mixed culture as shown by a significant increase in the ratio of branch biomass to total above-ground biomass in the CF compared to the NF treatment and a significant effect on growth in mixture was only observed among the CF treatments (Fig. 4C and Table 3).

3.5. Root biomass

In general, a greater root biomass expressed as root dry mass/soil dry mass was observed in Viburnum as compared to beech (Fig. 5). A significant negative ozone effect on root biomass was observed on Viburnum, whereas beech showed a slight increase in root biomass in the NF compared to the CF treatment, although it was not statistically significant (Fig. 5). Root biomass in the mixed culture (roots of beech and Viburnum combined) showed a similar negative ozone effect as with Viburnum, but the difference was not statistically significant (Fig. 5).

4. Discussion

The observed difference between monoculture and mixture in terms of the development of ozone-induced visible foliar injury

Table 3
Probabilities of main effects of ozone (O_3) and competition (intraspecific vs. interspecific) (mixture) for mean above-ground biomass, branch biomass, branch biomass/above-ground biomass, seedling height, and percentage of total leaf area injured for seedlings of beech (*F. sylvatica*) and Viburnum (*V. lantana*) in 2003 and 2004

	Above-ground biomass	Branch biomass	Branch biomass/above-ground biomass	Height		Foliar injury	
	2004	2004	2004	2003	2004	2003	2004
Beech							
O_3	0.573	0.704	0.961	0.366	0.465	**<0.0001**	**<0.0001**
Mixture	**0.003**	**0.005**	0.495	0.999	0.212	0.898	**0.032**
O_3 × mixture	0.892	0.965	0.896	0.176	0.853		
Viburnum							
O_3	0.877	0.280	**0.016**	0.702	0.859	**<0.0001**	**<0.0001**
Mixture	**0.011**	**0.006**	0.244	**0.002**	**0.020**	0.238	**0.001**
O_3 × mixture	0.284	**0.013**	0.106	0.908	0.823		

Values in bold indicate significant differences at the 0.05 level.

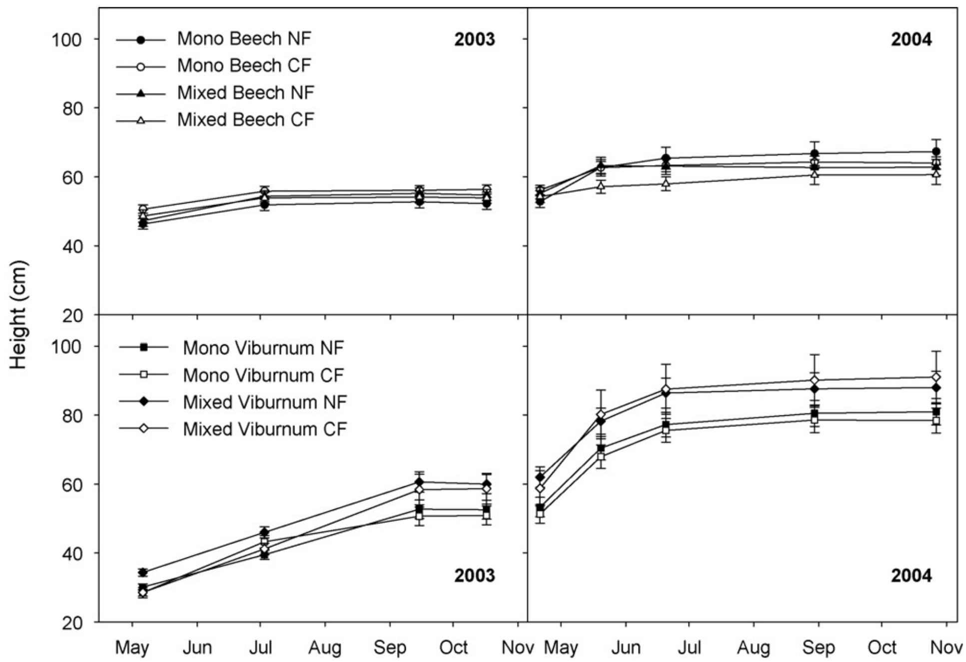

Fig. 3. Mean and standard error of seedling heights for beech (*F. sylvatica*) and Viburnum (*V. lantana*) grown in non-filtered (NF) and charcoal-filtered (CF) air treatments in monocultures and mixed cultures during 2003 and 2004.

and growth of beech and Viburnum seedlings evolved over the course of the 2003 and 2004 growing seasons as changes in canopy structure and the subsequent changes in physiology of the two species took place. Both beech and Viburnum exhibited foliar symptoms consistent with those observed during previous studies within the OTC facility (Skelly et al., 1998, 1999; VanderHeyden et al., 2001; Innes et al., 2001; Novak et al., 2003, 2005). In 2003, the percentage of symptomatic plants was similar among species with the exception of beech growing in the mixed culture. The timing of injury onset was also similar among species, but was approximately 1 month earlier in the mixed culture than in the monoculture for both species (Table 2). Viburnum has been previously characterized as more ozone sensitive than beech in respect to foliar injury (VanderHeyden et al., 2001), however, beech showed a greater percentage of total leaf area injured than Viburnum in 2003 (Fig. 2). Low values for total leaf area injured on Viburnum during the first year of transplanting have been observed during previous experiments within the OTC facility and were attributed to stresses involved with initial planting and establishment of the Viburnum seedlings (VanderHeyden et al., 2001; Novak et al., 2003), which were generally smaller than the beech seedlings. In addition, the extreme climatic conditions of 2003 may have also contributed to the lower injury values by limiting stomatal conductance and consequently decreasing stomatal uptake of ozone even though mean ozone concentrations were considerably higher in 2003 than in 2004.

In 2004, seedlings of both species were well established and the canopies were well developed. We found contrasting effects of interspecific competition on the injury development in Viburnum and beech seedlings. Viburnum was more sensitive to ozone when grown in mixture as compared to monoculture in terms of both percent total leaf area injured as well as the percentage of individuals showing ozone-induced injury, whereas, beech became less sensitive to ozone in the mixture with a considerably lower percentage of individuals showing injury as well as a lower percentage of total leaf area injured in comparison to beech grown in the monoculture (Fig. 2). In reality, ozone concentrations may vary considerably within the canopy, as recently shown for a forest stand (Karlsson et al., 2006) and intact grassland (Jäggi et al., 2006), and thus species growing at different levels within the canopy may experience much different ozone concentrations than observed above the canopy. Although specific measurements were not made at different heights within the canopy of the current OTC study, we expect that ozone concentrations were relatively similar throughout the canopy due to the constant circulation of air provided by the OTC fan system as well as the canopy structure, which has almost no green biomass at the soil surface. Instead, we expect that the observed differences in injury severity between beech in mixture versus monoculture were more likely due to differences in light intensity as a result of shading from the heterogeneous canopy structure created by dominant Viburnum individuals in 2004. Furthermore, the heterogeneous canopy structure would also explain the greater occurrence of injury on Viburnum in mixture as those dominant individuals may have experienced greater light exposure as a result of fewer stems. This is in agreement with Davison et al. (2003) who measured ozone concentrations, stomatal conductance and photosynthetically active radiation (PAR) in stands of cutleaf cone flower (*Rudbeckia laciniata* L.) growing along a trail as well as in the forest and found that the variation in symptom expression between the different stands was more likely due to variations in light within the two canopies rather than differences in ozone flux. However, in contrast to the cone flower study, we observed considerably lower leaf gas exchange

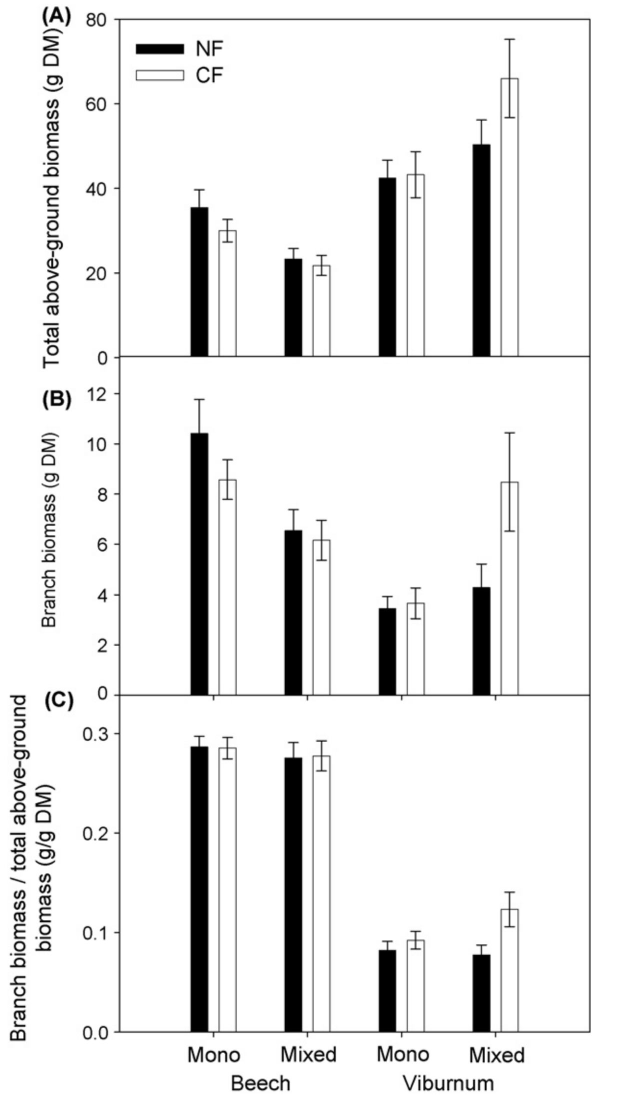

Fig. 4. Mean and standard error of total above-ground biomass (A), mean branch biomass (B), and branch biomass/total above-ground biomass (C) at the end of 2004 for seedlings of beech (*F. sylvatica*) and Viburnum (*V. lantana*) grown in non-filtered (NF) and charcoal-filtered (CF) air treatments in monoculture and mixed cultures during 2003 and 2004.

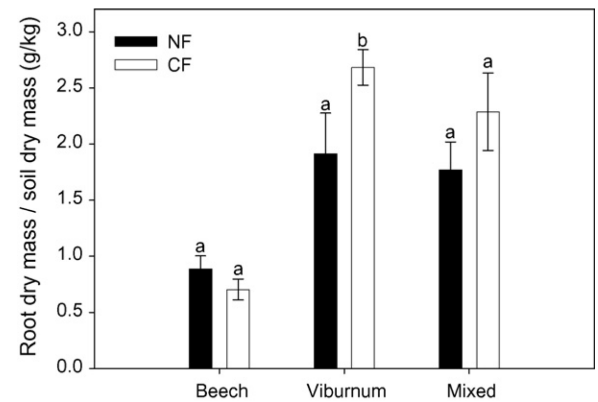

Fig. 5. Mean and standard error of root biomass (root dry mass/soil dry mass) at the end of 2004 for seedlings of beech (*F. sylvatica*) and Viburnum (*V. lantana*) and the mixture of the two species (mixed) grown in non-filtered (NF) and charcoal-filtered (CF) air treatments during 2003 and 2004. Different lower case letters indicate a significant difference between treatments at the 0.05 level for each plantation type.

rates in beech (especially stomatal conductance) in the mixture compared to the monoculture during the first half of the growing season (data not shown), which was most likely a result of lower light conditions experienced by beech individuals (Fredericksen et al., 1996). This would in turn result in lower ozone uptake and consequently, less severe injury expression as observed in beech grown in mixture with Viburnum. Nevertheless, negative effects on growth of beech due to ozone were not detected, contrasting with other studies that found decreased growth following ozone exposure and under low light conditions (Topa et al., 2001; Tjoelker et al., 1993).

In general, the results of this study support our hypothesis as Viburnum appeared to be a stronger competitor than beech as reflected by a greater ability to accumulate above-ground biomass and an overall greater height growth at the end of the 2-year experiment (Figs. 3 and 5). Significant negative effects of interspecific competition on total above-ground biomass accumulation in beech seedlings were observed, whereas, Viburnum seedlings appeared to benefit from growing in mixture, showing increases both in total above-ground biomass accumulation and height growth in comparison to the monoculture. Beech was also found to be a weaker competitor than spruce in a 2-year phytotron experiment, which exposed juvenile beech and spruce growing under inter- and intra-specific competition to enhanced ozone and CO_2 concentrations (Grams et al., 2002; Liu et al., 2004; Kozovits et al., 2005a,b). The stronger competitive ability of spruce was attributed to its greater above-ground space sequestration efficiency in comparison to beech (Grams et al., 2002; Kozovits et al., 2005b). Our results appear to support this general hypothesis as Viburnum exceeded beech in height growth by approximately 30 cm (Fig. 3), resulting in considerable shading of beech. Furthermore, Viburnum showed adjustments in biomass partitioning as the branch biomass per total biomass ratio increased when grown in mixture with beech (Fig. 4C). This suggests that apical growth may be stimulated in a competitive environment while the lateral buds suffer from the apical dominance. However, this was mainly the case for Viburnum growing in the CF treatment, whereas, ozone in ambient air seemed to counteract this effect in the NF treatment. This is consistent with other studies that have also found that ozone may alter crown structure (Braun et al., 2007; Ling, 2003; Dickson et al., 2001). Although not statistically significant, a similar trend was observed in total above-ground biomass (Fig. 4A), which suggests that ozone may repress the ability of Viburnum to benefit from interspecific competition with beech in terms of above-ground biomass accumulation. Beech showed no significant differences in the ratio of branch biomass to total above-ground biomass in response to either ozone or growth in mixture (Fig. 4C), indicating that biomass distribution patterns remained consistent despite significant changes in overall biomass accumulation. In this respect, beech proved to be less flexible in its ability to compete with Viburnum, which is likely due to its relatively slower growth and limiting determinant growth pattern. On the contrary, the greater ability of Viburnum to acquire above-ground space was likely driven by its inherently faster growth rate and generally greater plasticity in growth pattern as compared to beech.

In terms of below-ground biomass, Viburnum had a greater root biomass (root dry mass/soil dry mass) than beech (Fig. 5) when comparing monocultures of both species. Although not statistically significant, root biomass in beech tended to be stimulated under ozone stress, as was the case with above-ground biomass. This trend is contradictory in respect to other studies that found a greater reduction in root biomass as compared to shoots (Rebbeck and Scherzer, 2002; Paludan-Müller et al., 1999). Although total root biomass could not be determined, the results for relative root biomass indicate no change in biomass partitioning between shoots and roots (Figs. 5A, B and 6). On the contrary, a significant negative ozone effect on root biomass was observed in Viburnum. Interestingly, no significant ozone effect on above-ground biomass was observed for Viburnum in monoculture, suggesting that ozone may decrease relative biomass partitioning to the roots without any significant effects observed in above-ground biomass, which is in agreement with studies on other species (*cf.* Andersen, 2003). Although not significant, root biomass in the mixed culture showed a similar negative ozone effect as with Viburnum. This trend was likely driven by Viburnum root biomass rather than beech root biomass, however, the question as to how root biomass was affected as a result of ozone stress and competition between the two species remains unanswered.

Although significant effects of ozone and effects on growth in mixture were observed during this 2-year experiment, a longer experimental duration may have resulted in more significant ozone effects as well as stronger competitive interactions. The potential for significant ozone effects beyond the typical 2–3-year experiment duration, as recently shown for semi-natural, old grassland by Volk et al. (2006), emphasizes the need for more long-term experiments conducted under relevant field conditions (e.g., with interspecific competition) so that we can better assess the potential impacts of ozone in natural systems (Matyssek and Sandermann, 2003; Bassin et al., 2007). Furthermore, it is important to note that the results of this OTC current study were observed under ambient (NF) ozone concentrations as they naturally occur in southern Switzerland, whereas an additional treatment with elevated ozone under the given conditions may have resulted in even stronger ozone effects and competitive interactions.

5. Conclusions

In conclusion, these results support our initial hypothesis as Viburnum was a stronger competitor than beech over the course of this 2-year study. Seedlings of Viburnum benefited from interspecific competition in terms of both height growth and above-ground biomass accumulation at the expense of beech seedlings, which showed significantly reduced growth in the mixture as compared to the monoculture. However, as this was only the case for Viburnum growing in the CF treatment, ozone seemed to counteract the beneficial effect of interspecific competition on above-ground biomass accumulation in Viburnum, while at the same time decreasing relative biomass allocation to roots. Foliar sensitivity of the two species was also altered under interspecific competition suggesting that results based on seedlings of single species grown in monocultures may significantly over- or under-estimate foliar sensitivity to ozone. These results demonstrate that competition is an important factor affecting plant responses to ozone stress, but the direction and severity of these effects depend on the interacting species.

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References

Andersen, C.P., 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol. 157, 213–228.

Andersen, C.P., Hogsett, W.E., Plocher, M., Rodecap, K., Lee, E.H., 2001. Blue wild-rye grass competition increases the effect of ozone on ponderosa pine seedlings. Tree Physiol. 21, 319–327.

Barbo, D.N., Chappelka, A.H., Somers, G.L., Miller-Goodman, M.S., Stolte, K., 2002. Ozone impacts on loblolly pine (*Pinus taeda* L.) grown in a competitive environment. Environ. Pollut. 116, 27–36.

Bassin, S., Volk, M., Fuhrer, J., 2007. Factors affecting the ozone sensitivity of temperate European grasslands: an overview. Environ. Pollut. 146, 678–691.

Braun, S., Schindler, C., Rihm, B., Flückiger, W., 2007. Shoot growth of mature *Fagus sylvatica* and *Picea abies* in relation to ozone. Environ. Pollut. 146, 624–628.

Davison, A.W., Neufeld, H.S., Chappelka, A.H., Wolff, K., Finkelstein, P.L., 2003. Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower *Rudbeckia laciniata* L. Environ. Pollut. 125, 61–70.

Dickson, R.E., Coleman, M.D., Pechter, P., Karnosky, D., 2001. Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. Environ. Pollut. 115, 319–334.

Fredericksen, T.S., Steiner, K.C., Skelly, J.M., Joyce, B.J., Kolb, T.E., Savage, J.E., 1996. Light environment alters ozone uptake per net photosynthesis rate in black cherry trees. Tree Physiol. 16, 485–490.

Grams, T.E.E., Kozovits, A.R., Reiter, I.M., Winkler, J.B., Sommerkorn, M., Blaschke, H., Häberle, K.-H., Matyssek, R., 2002. Quantifying competitiveness in woody plants. Plant Biol. 4, 153–158.

Horsfall, J.G., Barratt, R.W., 1945. An improved grading system for measuring plant disease. Phytopathology 35, 655.

Innes, J.L., Skelly, J.M., Schaub, M., 2001. Ozone and Broadleaved Species: A Guide to the Identification of Ozone-induced Foliar Injury/Ozon, Laubholz- und Krautpflanzen: Ein Führer zum Bestimmen von Ozonsymptomen. Birmensdorf, Eidgenössische Forschungsanstalt WSL. Bern, Stuttgart, Wien, Haupt, 136 pp.

Jäggi, M., Ammann, C., Neftel, A., Fuhrer, J., 2006. Environmental control of profiles of ozone concentration in a grassland canopy. Atmos. Environ. 40, 5496–5507.

Karlsson, P.E., Hansson, M., Höglund, H.-O., Pleijel, H., 2006. Ozone concentration gradients and wind conditions in Norway spruce (*Picea abies*) forests in Sweden. Atmos. Environ. 40, 1610–1618.

Kolb, T.E., Fredericksen, T.S., Steiner, K.C., Skelly, J.M., 1997. Issues in scaling tree size and age responses to ozone: a review. Environ. Pollut. 98, 195–208.

Kollmann, J., Grubb, P.J., 2002. *Viburnum lantana* L. and *Viburnum opulus* L. (*V. lobatum* Lam., *Opulus vulgaris* Borkh.). J. Ecol. 90, 1044–1070.

Kozovits, A.R., Matyssek, R., Blaschke, H., Göttlein, A., Grams, T.E.E., 2005a. Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated CO_2 and O_3 levels throughout two subsequent growing seasons. Glob. Change Biol. 11, 1387–1401.

Kozovits, A.R., Matyssek, R., Winkler, J.B., Göttlein, A., Blaschke, H., Grams, T.E.E., 2005b. Above-ground space sequestration determines competitive success in juvenile beech and spruce trees. New Phytol. 167, 181–196.

Ling, K.A., 2003. Using environmental and growth characteristics of plants to detect long-term changes in response to atmospheric pollution: some examples from British beechwoods. Sci. Total Environ. 310, 203–210.

Liu, X., Kozovits, A.R., Grams, T.E.E., Blaschke, H., Rennenberg, H., Matyssek, R., 2004. Competition modifies effects of enhanced ozone/carbon dioxide concentrations on the carbohydrate and biomass accumulation in juvenile Norway spruce and European beech. Tree Physiol. 24, 1045–1055.

Matyssek, R., Innes, J.L., 1999. Ozone—a risk factor for trees and forests in Europe? Water Air Soil Pollut. 116, 199–226.

Matyssek, R., Sandermann, H., 2003. Impact of ozone on trees: an ecophysiological perspective. Progress in botany, vol. 64. Springer-Verlag, Heidelberg, pp. 349–404.

McDonald, E.P., Kruger, E.L., Riemenschneider, D.E., Isebrands, J.G., 2002. Competitive status influences tree-growth responses to elevated CO_2 and O_3 in aggrading aspen stands. Funct. Ecol. 16, 792–801.

Novak, K., Skelly, J.M., Schaub, M., Kräuchi, N., Hug, C., Landolt, W., Bleuler, P., 2003. Ozone air pollution and foliar injury development on native plants of Switzerland. Environ. Pollut. 125, 41–52.

Novak, K., Schaub, M., Fuhrer, J., Skelly, J.M., Hug, C., Landolt, W., Bleuler, P., Kräuchi, N., 2005. Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plant species. Environ. Pollut. 136, 33–45.

Paludan-Müller, G., Saxe, H., Leverenz, J.W., 1999. Responses to ozone in 12 provenances of European beech (*Fagus sylvatica*): genotypic variation and chamber effects on photosynthesis and dry matter partitioning. New Phytol. 144, 261–273.

Pye, J.M., 1988. Impact of ozone on the growth and yield of trees. J. Environ. Qual. 17, 347–360.

Rebbeck, J., Scherzer, A.J., 2002. Growth responses of yellow-poplar (*Liriondendrn tulipifera* L.) exposed to 5 years of O_3 alone or combined with elevated CO_2. Plant Cell Environ. 25, 1527–1537.

SAS Institute Inc., 1999. SAS Software Version 8. SAS Institute Inc., Cary, NC.

Skelly, J.M., Innes, J.L., Snyder, K.R., Savage, J.E., Hug, C., Landolt, W., Bleuler, P., 1998. Investigations of ozone-induced injury in forests of southern Switzerland: field surveys and open-top chamber experiments. Chemosphere 36, 995–1000.

Skelly, J.M., Innes, J.L., Savage, J.E., Snyder, K.R., Vanderheyden, D., Zhang, J., Sanz, M.J., 1999. Observation and confirmation of foliar ozone symptoms of native plant species of Switzerland and southern Spain. Water Air Soil Pollut. 116, 227–234.

Tjoelker, M.G., Volin, J.C., Oleksyn, J., Reich, P.B., 1993. Light environment alters response to ozone stress in seedlings of *Acer saccharum* Marsh. and hybrid *Populus* L. I. In situ net photosynthesis, dark respiration and growth. New Phytol. 124, 627–636.

Topa, M.A., Vanderklein, D.W., Corbin, A., 2001. Effects of elevated ozone and low light on diurnal and seasonal carbon gain in sugar maple. Plant Cell Environ. 24, 663–677.

VanderHeyden, D.J., Skelly, J.M., Innes, J.L., Hug, C., Zhang, J., Landolt, W., Bleuler, P., 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environ. Pollut. 111, 321–331.

Volk, M., Bungener, P., Contat, F., Montani, M., Fuhrer, J., 2006. Grassland yield declined by a quarter in 5 years of free air fumigation. Glob. Change Biol. 1, 74–83.

Zhang, J., Ferdinand, J.A., VanderHeyden, D.J., Skelly, J.M., Innes, J.L., 2001. Variation in gas exchange within native plant species of Switzerland and relationships with ozone injury: an open-top experiment. Environ. Pollut. 113, 117–185.